

Pseudomonas Aeruginosa-Fecal Coliform
Relationships in Estuarine and Fresh
Recreational Waters

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16. ABSTRACT This study has shown that <u>P. aeruginosa</u> cannot be used as the basis of water standards for the prevention of enteric disease during the recreational use of surface waters. However, <u>P. aeruginosa</u> determinations, when used in conjunction with the assay of fecal coliforms or some other indicator organism, could be of considerable value in the development of criteria for recreational waters. <u>P. aeruginosa</u> determinations could provide useful information relative to the nature of pollution sources. The finding of high <u>P. aeruginosa</u> densities coincident with high fecal coliform levels (PA:FC ratios >20) should signal the possibility that the source of the fecal coliforms may not be fecal wastes.		
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***Pseudomonas aeruginosa*-fecal coliform relationships in estuarine and fresh recreational waters**

VICTOR J. CABELLI, HARRIET KENNEDY, AND MORRIS A. LEVIN

MOST STATES HAVE ESTABLISHED water quality standards for recreational waters based on the coliform index,¹ even though there is no conclusive evidence of a correlation between coliform levels in recreational waters and the incidence of illness in populations bathing therein.^{2,3} The absence of conclusive evidence could be real in that there may be little if any hazard of enteric infection to bathers even when the recreational waters used are grossly polluted with fecal wastes. Moore *et al.*⁴ concluded that, in the absence of gross contamination with visible fecal material, there is a negligible health hazard associated with bathing in wastewater-polluted marine waters. Stevenson⁵ did observe two instances that suggested a correlation between gastrointestinal disease and coliform densities in freshwaters used for bathing. He noted that the evidence was far from conclusive, however.

It is the present authors' view that there are measurable health hazards associated with swimming in waters polluted with fecal wastes but that limitations in the design of previous studies have precluded detection of these hazards. For example, in none of the previous studies, including those of Stevenson,⁵ was swimming or bathing defined so that the incidence of illness was measured in the population actually at risk, that is, individuals with significant exposure of their upper body orifices to the water. Furthermore, many workers have questioned the validity of total coliform densities as an index of fecal pollution.^{2,3,6} Several states use fecal coliform standards; in fact, the proposed National Technical Advisory Council (NTAC) standard is based

on fecal coliform densities in water.⁷ There are, however, no data correlating fecal coliform densities to enteric disease in bathing populations.

Among the other organisms proposed as possible indicators of bathing water quality are fecal streptococci and *Pseudomonas aeruginosa*.^{8,9} The latter organism has some advantages over coliforms as an indicator of human fecal pollution: it is primarily associated with human as opposed to animal feces¹⁰ and has better survival characteristics than the organisms of the coliform group.¹¹ Another justification for the classification of bathing waters based on *P. aeruginosa* densities is the hazard of swimming-associated ear infections, primarily otitis externa. Stevenson⁵ found that ear and eye complaints accounted for more than one-half of the illnesses associated with swimming, particularly in swimming pools. The association of *P. aeruginosa* infections of the external ear with swimming is so common that the term "swimmer's ear" has been used to describe such cases.¹² Favero *et al.*¹³ and Hoadley¹⁰ have suggested that restrictions be placed on *P. aeruginosa* densities in bathing waters because of the association of this organism with ear infections and its presence in swimming pools and natural bathing areas.

A number of studies have been conducted to quantitate *P. aeruginosa* levels in recreational waters^{9,13} and, in one case, to relate the levels to the incidence of ear infections among the bathers.³ These workers³ were unable to demonstrate a relationship between the *P. aeruginosa* levels observed and the rate of ear in-

fections. In the above studies, quantification of *P. aeruginosa* was attempted by using a most probable number (MPN) procedure.¹⁴ The development of a membrane filter procedure, which is more precise and accurate than the MPN method for the enumeration of this organism,¹⁵ calls for a re-examination of *P. aeruginosa* densities in surface waters and of their relationship to the incidence of ear infections among bathers. The present study was designed to determine the relationship of *P. aeruginosa* densities to fecal coliform levels in surface waters located at varying distances from known sources of pollution.

MATERIALS AND METHODS

Survey areas. The water samples used to study *P. aeruginosa*-fecal coliform relationships were collected from estuarine and fresh surface waters at two areas in Rhode Island. The areas were Pawtuxet River-Providence River-upper Narragansett Bay and Saugatucket River-Point Judith Pond. Sampling was conducted on a seasonal basis at monthly intervals during the winter, spring, and summer of 1971. A brief description of the sampling stations, some of the sources of pollution (as given on a map published by the State of Rhode Island in 1967¹⁶), and the sampling regimen at each area are as follows:

Area 1. This area (Figure 1) contained five freshwater sampling stations on the Pawtuxet River (P1 to P5) and seven estuarine stations on the Providence River and upper Narragansett Bay (N1 to N7). The Pawtuxet River empties into Providence River, which, in turn, empties into Narragansett Bay. Three of the seven estuarine stations were located above the mouth of the Pawtuxet River. Raw and treated sanitary wastes from a number of municipal sewerage systems and the effluents from several textile finishing plants are discharged into this complex. Seven samples were collected from each station, at least two during each of the seasonal studies.

Area 2. A series of ten sampling stations (S1 to S10) extending from the headwaters of the river through the pond and into Block Island Sound were established and sampled (Figure 1). The water at Stations S1 to S4 is fresh. Thereafter, the salinity of the water increases from <5 parts per thousand at Stations S5 and S6 to about 25 parts per thousand at Station S10. The salinities at Stations S5 to S10 vary with tidal activity and streamflow. Five samples were collected from each station, at least one during each of the three seasons studied. In contrast to Area 1, there is no discharge of municipal wastewater treatment plant effluents into the Saugatucket River or Point Judith Pond. The 1967 map notes two sources of pollution: a textile finishing plant and private wastewater lines in the vicinity of the town of Wakefield (Figure 1). In addition, there are marinas at several locations along the length of Point Judith Pond.

Area 3. The findings from the Rhode Island aspect of the study were then applied to data on *P. aeruginosa* and fecal coliform densities collected from six bathing beaches located in the Evanston-North Shore area of Lake Michigan (Figure 2). These beaches were sampled daily, except weekends, during late June, July, and August.

Sampling and assay methods. Samples were collected and assayed for fecal coliform densities as stated in "Standard Methods for the Examination of Water and Wastewater".¹⁷ Fecal coliform levels were estimated by using the MPN procedure and the mrc technique with the Rhode Island and Lake Michigan samples, respectively. *P. aeruginosa* levels were determined by using the membrane filter technique of Levin and Cabelli.¹⁵

RESULTS

The *P. aeruginosa* and fecal coliform levels at the various sampling stations are given in Table I; the values shown are the geometric means of the monthly recoveries. The *P. aeruginosa* and fecal coliform densities decreased as the distance between

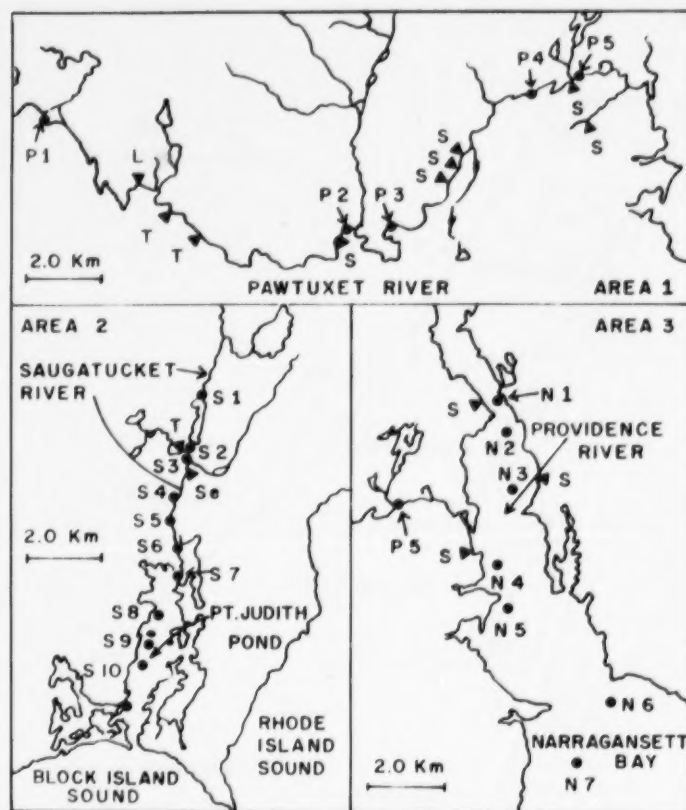


FIGURE 1.—Location of sampling stations and some of the sources of pollution in the Rhode Island study areas. Sampling stations: P, N, and S. Sources of pollution: L (laundry), T (textile finishing plant), and S (wastewater treatment plant).

the sampling stations and the major sources of fecal pollution (Figure 1) increased. The decreases did not parallel each other, however. That is, the ratios of *P. aeruginosa*:fecal coliform densities $\times 100$ (PA:FC) were greatest at those stations (N6, N7, and S7 to 10) farthest from the pollution sources. PA:FC ratios of less than 2.5 were associated with immediate sources of pollution. The exception was Station S3, where a high fecal coliform density was accompanied by a relatively high *P. aeruginosa* level. This suggested the possibilities that the source of the organisms was not sanitary wastes and that the fecal

coliforms were not of fecal origin but rather were associated with some specific activity at a textile mill located just upstream from the station; this situation will be outlined in a future paper. The *P. aeruginosa* level comparable to the proposed NTAC fecal coliform standard of 200/100 ml for direct contact recreational waters was calculated from a plot of the mean *P. aeruginosa* density for each station against its respective mean fecal coliform level (Figure 3); it was 12/100 ml.

The suggestion that the fecal coliform and *P. aeruginosa* levels did not parallel each other was confirmed by plotting the

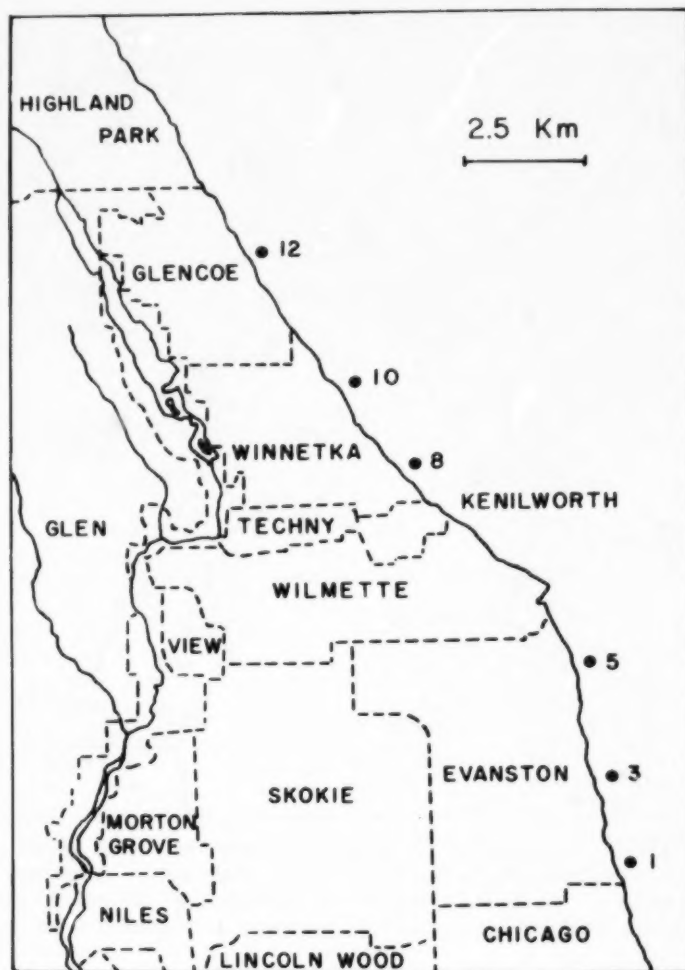


FIGURE 2.—Sampling stations along north shore of Lake Michigan.

PA:FC ratio for each sample against its fecal coliform density. If the levels of the two organisms paralleled each other, the points should have scattered about a horizontal line. Analysis of the regression of the transformed (log) ratios and fecal coliform densities showed that the slopes of the lines (ratio on fecal coliform density) for the estuarine and freshwater samples (Figure 4) were significantly negative at the $P = 0.005$ level; that is, the ratios in-

creased as the fecal coliform densities decreased. The regression coefficients of the freshwater as compared with the estuarine and the summer as compared with the winter and spring samples seemed to be different. Statistically significant differences even at the $P = 0.1$ level were not obtained, however, possibly because of excessive sample variability: the standard errors of both the regression coefficients and the regression estimates were rela-

FECAL COLIFORM RELATIONSHIPS

TABLE I.—*Pseudomonas aeruginosa* and Fecal Coliform Densities in Fresh and Estuarine Waters in Rhode Island

Sam- pling Station*	Type of Water	Mean recovery/100 ml		<i>Pseudomonas</i> <i>aeruginosa</i> : fecal coliforms (PA:FC × 100)
		<i>Pseudomonas</i> <i>aeruginosa</i> †	Fecal Coliforms†	
P1	Fresh	<1	3.1	
2		140	6,700	2.0
3		71	2,400	3.0
4		62	2,000	3.1
5		66	2,600	2.5
N1	Estuarine	24	1,100	2.1
2		24	1,500	1.6
3		18	1,400	1.3
4		27	490	5.5
5		17	600	2.8
6		7.7	63	12
7		4.2	47	9.0
S1	Fresh	2.1	49	4.2
2		7.9	200	4.0
3		2,100	8,000	27
4		190	2,900	6.6
5		210	8,800	2.3
6	Estuarine	170	4,300	3.8
7		8.6	140	6.1
8		8.5	27	31
9		5.9	57	10
10		2.6	19	14.0

* Sampling stations shown in Figure 1.

† Number of samples: P and N stations, 6 to 7; S stations, 4 to 5.

tively high. The PA:FC values for the samples from three of the freshwater stations (S1, S2, and S3) seemed to be distributed differently from the remaining values. The ratios for the S3 samples were very high relative to their fecal coliform densities, and those for the S1 and S2 samples were relatively low.

The possibility of using *P. aeruginosa* as an indicator of fecal pollution or, in conjunction with fecal coliform assays, as a means of determining the immediacy of a pollution source was examined in the course of a study to evaluate the mPA method at several bathing beaches along Lake Michigan. At the time of the study, it was known that sanitary pollution as evidenced by high fecal coliform levels was carried to the beaches coincident with north to northeast winds. High coliform levels have been reported at locations north of the area being studied.¹⁸ Fecal pollution entering the beaches between Stations 1 and 12 (Figure 2) or carried to the beaches from sources to the south had not been detected. It may be seen (Figure 4) that, as was observed with the

Rhode Island data, the PA:FC ratios increased as the fecal coliform levels decreased. The slope of the regression line (PA:FC ratio on fecal coliform density) was significantly different from the one obtained from the saltwater samples collected in Rhode Island, and the fecal coliform levels associated with a PA:FC ratio of 2.5 were about one order of magnitude less than those obtained with the Rhode Island data.

The comparability of a *P. aeruginosa* density of 12/100 ml to a fecal coliform level of 200 was not verified. That is, *P. aeruginosa* densities in excess of 10/100 ml frequently were associated with fecal coliform levels considerably less than 200/100 ml (Table II). Conversely, fecal coliform levels in excess of 200/100 ml were associated at times with very low *P. aeruginosa* densities (Table III). The former condition occurred most frequently on the first or second day following a north wind and as the distance south from Station (beach) 12 increased. The latter situation was observed at Stations 10 and 12

TABLE II.—Fecal Coliform Densities Coincident with *Pseudomonas aeruginosa* Levels of 10/100 ml or More

Date (1971)	Day*	Wind Direction (in degrees)	Station	Recovery/100 ml of	
				<i>Pseudo- monas</i> <i>aeru- ginosa</i>	Fecal Coli- forms†
6/22	N + 1	210	12	19	170
7/20	N + 1	240	5	32	52
			8	10	160
			10	58	120
			12	18	85
7/21	N + 2	225	1	14	10
			3	27	6
			8	21	<2
7/22	>N + 2	225	12	32	245
7/26	N + 2	315	5	21	4
8/12	N + 1	210	1	16	10
8/16	N	0	5	18	12
8/26	N + 1	315	1	19	14
			3	14	110
			8	13	70
			10	10	200

* N—day of north wind.

† Fecal coliforms by mFC procedure.

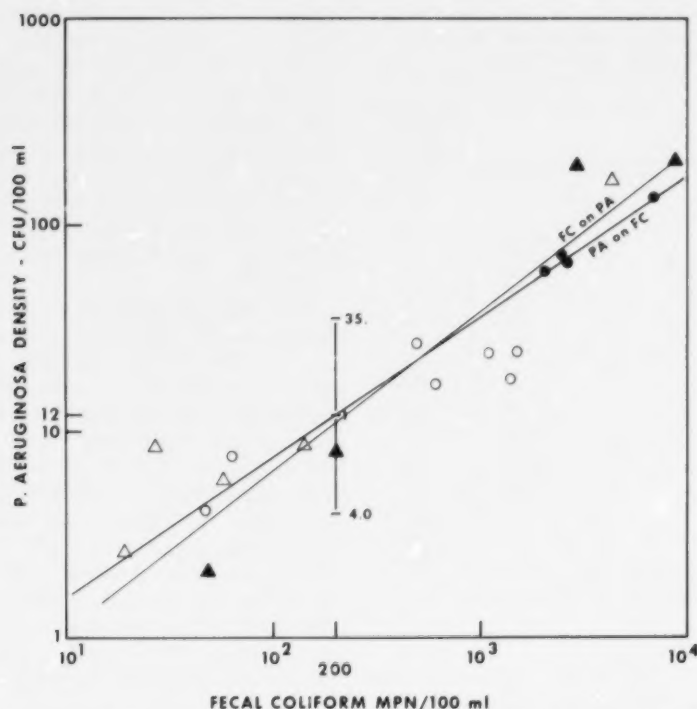


FIGURE 3.—Relationship of *Pseudomonas aeruginosa* to fecal coliform densities in fresh and estuarine surface waters in Rhode Island. (Values given are the geometric means of the monthly recoveries.) ●—freshwater, Pawtuxet River; ▲—freshwater, Saugatucket River; ○—estuarine water, Providence River-Narragansett Bay; △—estuarine water, Point Judith Pond. Lines calculated by regression analysis of variance. Data from Station S3 not used in calculating regression line. *Pseudomonas aeruginosa* density comparable to fecal coliform most probable number of 200:100 ml (National Technical Advisory Council standard for recreational waters) = 12:100 ml with 95 percent CI of 4.0 to 35.

on the day of a north wind. The high *P. aeruginosa* levels associated with low fecal coliform densities on the two days following a north wind, when compared with the low *P. aeruginosa* densities associated with high fecal coliform densities at Stations 10 and 12 on the day of a north wind, suggested that *P. aeruginosa* multiplied in the water at the beaches being studied. This was examined by comparing *P. aeruginosa* levels, fecal coliform

densities, and PA:FC ratios by station and by day following a north wind (Figure 5). The geometric means of the recoveries and of the PA:FC ratios were calculated for each station at each "day category": N (day of north wind), N + 1, N + 2, >N + 2. Densities below the sensitivity of the assay method, 0.4/100 ml for *P. aeruginosa* and 1/100 ml for fecal coliforms, were arbitrarily set at those values and were used in calculating the mean

FECAL COLIFORM RELATIONSHIPS

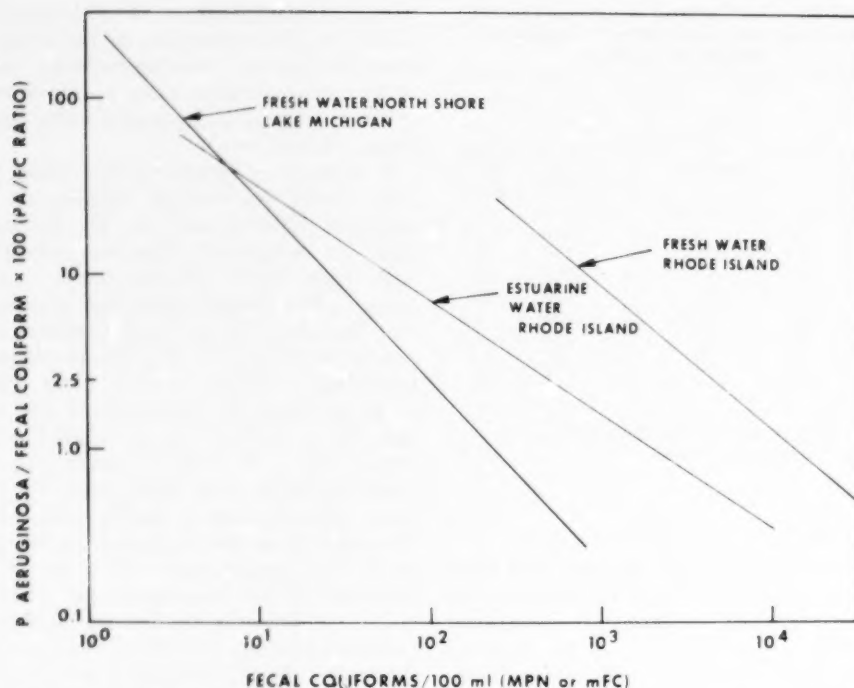


FIGURE 4.—Relationship of *Pseudomonas aeruginosa* to fecal coliform levels (PA:FC) as a function of the fecal coliform densities in samples collected in Rhode Island and from Lake Michigan. Lines were drawn from values obtained by regression analysis of the transformed (log) data.

densities. Ratios were not calculated when the *P. aeruginosa* density was $<1.6/100$ ml (4 colonies/filter when a 250-ml water sample was assayed), however, and the fecal coliform density was $<4/100$ ml (4 colonies/filter when a 100-ml sample was assayed) in order to avoid using ratios in which both the numerator and denominator were relatively unreliable because of a small number error. The reciprocals (r) of the PA:FC ratios are given in Figure 5 for better visual comparison with the fecal coliform densities. It may be seen that, on Day N, the *P. aeruginosa* densities increased with the distance from the known pollution source to the north of Station 12. The levels also increased on the day following a north wind and then declined to a very low level by the third

day. This confirmed the multiplication of *P. aeruginosa*, probably in association with residual fecal pollution of the water at the beaches. The fecal coliform densities and (r) values decreased both with the distance south from Station 12 and with the time interval following a north wind, confirming the presence of a moderate pollution source immediately north of Station 12. The downward trend of the (r) ratios was reversed between Stations 5 and 3; however, the reversal was most marked in those samples collected more than 2 days after a north wind. This suggested the presence of a small "distant" pollution source(s) discharging into the lake in the vicinity of Station 3 and/or a very low level of pollution reaching Station 1 from a distant source south of this station.

TABLE III.—*Pseudomonas aeruginosa* Densities Coincident with Fecal Coliform Densities of 150/100 ml or More

Date (1971)	Day*	Wind Direction (in degrees)	Station	Recovery /100 ml of	
				Fecal Coliforms†	<i>Pseudomonas aeruginosa</i>
6/21	N	0	12	570	>1.6
6/22	N + 1	210	12	170	19
7/20	N + 1	170	8	160	10
7/22	>N + 2	225	12	250	32
8/11	N	45	10	150	2.8
	N	45	12	280	<1.6
8/16	N	0	8	180	9.2
8/26	N + 1	315	10	200	10

* N—day of north to northeast wind.

† Fecal coliforms on mFC.

DISCUSSION

It is quite clear from the data presented that, because of its poor reliability as an indicator of fecal pollution, *P. aeruginosa* of itself cannot be used as the basis of water standards for the prevention of enteric disease during the recreational use of surface waters. Even if workable standards could be developed to accommodate the geographic and seasonal effects on *P. aeruginosa* densities that were observed in the present study, the discharge of large numbers of *P. aeruginosa* from nonfecal sources—as suggested by the data from Station S3—and the multiplication of the organism in surface waters subject to low levels of nutrient pollution would argue against its use.

The above considerations notwithstanding, *P. aeruginosa* determinations, when used in conjunction with the assay of fecal coliforms or some other indicator organism, could be of considerable value in the development of criteria for recreational waters. At bathing beaches, such as those at Lake Michigan, subject to intermittent pollution because of rainfall, windshifts, and so forth, a temporary beach closure could be maintained until the *P. aeruginosa* densities return to a baseline level. This assumes that the dilution of those nutrients in wastewater that permit the limited and

transient multiplication of *P. aeruginosa* would parallel reductions in the levels of enteropathogenic microorganisms, especially viruses, better than a combination of the biological and physical reduction of fecal coliform levels.

P. aeruginosa determinations could provide useful information relative to the nature of pollution sources. The finding of high *P. aeruginosa* densities coincident with high fecal coliform levels (PA:FC ratios >20) should signal the possibility that the source of the fecal coliforms may not be fecal wastes. The results obtained from Station S3 are a case in point. Data to be presented in a future paper will confirm the suggestion that the high *P. aeruginosa* and fecal coliform densities in the water samples were attributable to non-fecal effluents from a textile mill. Furthermore, if, as was suggested by Hoadley *et al.*,¹⁰ *P. aeruginosa* is not a normal inhabitant of the gastrointestinal tract of lower animals, the finding of high fecal coliform densities (>1,000/100 ml) coincident with low *P. aeruginosa* levels (<1/100 ml) should suggest the possibility that the source of fecal pollution is animal rather than human.

Those very considerations that limit the use of *P. aeruginosa* as an indicator of fecal pollution—low *P. aeruginosa* densities associated with high fecal coliform levels near a source of fecal pollution and relatively high *P. aeruginosa* levels coincident with low fecal coliform levels at great distances from pollution source—could permit the PA:FC ratios to be used as a measure of the proximity of a pollution source and as a means of detecting low pollution levels arising from distant origins. The prediction of a small source carried to the vicinity of Station 3 on Lake Michigan was verified by a shoreline examination; a boat ramp and an outlet from a lagoon were found in the area of this station. The PA:FC ratios and, to a lesser extent, the fecal coliform densities suggested a very low level of pollution that, coincident with southerly winds, reached Station 1 from a distant origin to the south. This predicted source is compatible with

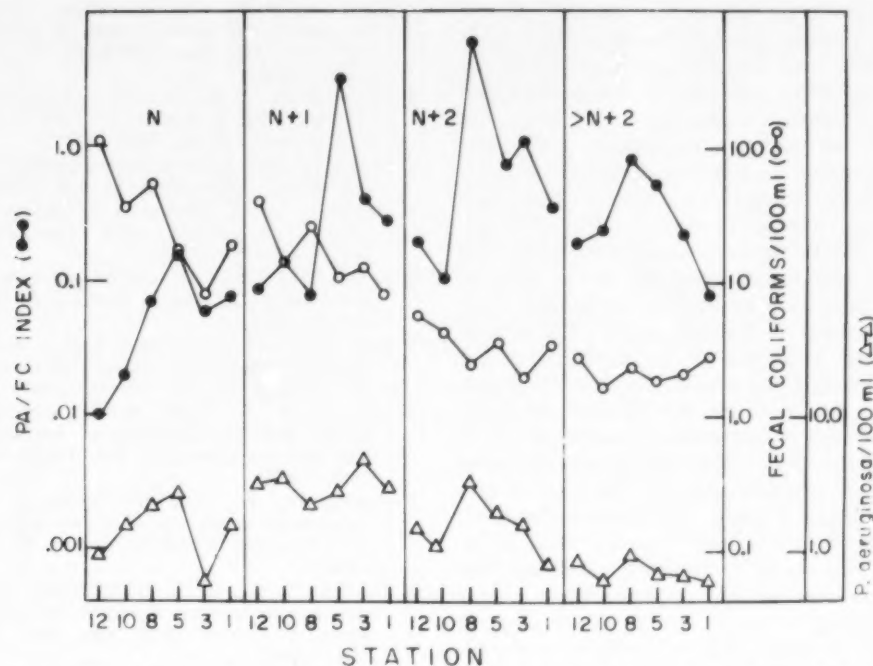


FIGURE 5.—*Pseudomonas aeruginosa* and fecal coliform densities and "r" ratios in waters at beaches along north shore of Lake Michigan. "r" = reciprocal of PA:FC; PA:FC = *Pseudomonas aeruginosa*/100 ml/fecal coliforms/100 ml $\times$ 100. N—day of north to northeast wind; abscissa—stations as indicated in Figure 2. Samples collected during June, July, and August. Data grouped by day category N, N + 1, N + 2, and $<N + 2$ and by station. Each point represents geometric mean of densities from 3 to 11 samples. Δ —*Pseudomonas aeruginosa*/100 ml; $\circ$ —fecal coliforms (mfc method)/100 ml; $\bullet$ —PA:FC index.

known pollution levels at Chicago beaches south of Station 1.^{3, 18} Additional studies are needed to test the validity of this approach, especially because the possibility exists that the higher ratios obtained at Stations 1 and 3 may have been artifacts. Foster *et al.*³ recently described the coliform and *P. aeruginosa* densities at three bathing beaches. With one exception, the *P. aeruginosa*:total coliform ratios calculated from their data were at least 5.0. It is reasonable to assume that the PA:FC ratios would have been still higher. Ratios such as these would suggest that the source of fecal pollution was at some distance from the beaches and/or that *P. aeruginosa*

was multiplying in the water. If either of the above possibilities were operative, the absence of swimming-related enteric illness would not be unexpected. In fact, it is precisely this type of situation in which a determination of the PA:FC ratio may be of value.

Although, as was noted previously, ear and eye infections caused by *P. aeruginosa* constitute a major hazard of swimming, there is no conclusive evidence demonstrating a cause and effect relationship of *P. aeruginosa* densities in the water to the incidence of these infections in swimming populations. Because about 15 percent of the human population have the organism

in the ear as part of the "normal" flora,¹² it is entirely possible that it is these individuals who actually are at risk, that the source of the organisms is endogenous, and that "trauma" to the ear canals associated with swimming (especially underwater swimming) and diving allows these organisms to invade the tissues. In fact, individuals who normally carry this organism may not be "normal" at all in the sense that they may already have lost one or more of the barriers that prevent the growth of *P. aeruginosa* in the ear canal. These individuals will have to be identified in the epidemiological studies required to determine the need for criteria and standards governing *P. aeruginosa* densities in recreational waters. The data presented herein suggest two situations for this type of epidemiological study. The observation that, at the Lake Michigan beaches, fecal pollution was carried in by north winds and that *P. aeruginosa* densities peaked 1 to 2 days following a north wind would suggest these or other beaches subject to fluctuating pollution for such a study. Secondly, high *P. aeruginosa* densities could be expected wherever recreational waters are subject to the discharge of nutrients such as those seen at Station S3.

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